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A New *Leptobrachium* (*Vibrissaphora*) from Laos (Anura: Megophryidae)

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Abstract: A new species of the subgenus *Vibrissaphora* in the genus *Leptobrachium* is described from eastern Laos, on the basis of molecular and morphological evidence. The new species differs from all members of the subgenus by lack of spines on upper lip, small size with adult male SVL of 55.2 mm, indistinct tympanum, first finger longer than fourth, inner metatarsal tubercle smaller than first toe, 2 $\frac{1}{3}$ phalanges of fifth toe unwebbed, dorsum nearly smooth and flanks with densely granulated skin, dorsum light brown with dark brown spots, flanks dark brown reticulated with cream spots, and abdomen cream with irregular brown markings. Classification of two subgenera in *Leptobrachium* is discussed.

Key words: Megophryidae; *Vibrissaphora*; Systematics; Laos; Biogeography

INTRODUCTION

The Southeast Asian megophryid genera *Leptobrachium* Tschudi, 1838 and *Vibrissaphora* Liu, 1945 are similar in general body shape, with broad head, long forelimbs, and short hindlimbs, but are clearly separated by the possession in *Vibrissaphora* and absence in *Leptobrachium* of nuptial spines on the upper labium in males during the breeding season (Liu, 1945). However, Tian and Jiang (1986) relegated *Vibrissaphora* to a subgenus of *Leptobrachium*, and Dubois and Ohler (1998) supported such classification because some characters other than horny spines overlap between the two genera. More recently, phylogenetic relationships among most members of the genus *Leptobrachium* (sensu lato)

have been nearly completely resolved through analyses of mitochondrial DNA genes (Matsui et al., 2010), where some of the continental species of *Leptobrachium* without spines on the upper lip formed a clade with members of *Vibrissaphora* having horny spines. Matsui et al. (2010) proposed recognizing two subgenera (*Leptobrachium* and *Vibrissaphora*) within the genus *Leptobrachium* like Tian and Jiang (1986) and Dubois and Ohler (1998), but the content of each subgenus is quite different. Namely, Matsui et al. (2010) proposed treating species of *Leptobrachium* nested in the mitochondrial DNA clade of *Vibrissaphora* as members of the subgenus *Vibrissaphora*, even though they lack horny spines on the upper lip, while previous authors split the two subgenera solely by the presence or absence of the spines.

In constructing a phylogenetic tree, Matsui et al. (2010) included an unidentified specimen

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from Laos, which was collected by the late Masataka Sato during his entomological survey there, and was donated to me for identification. Although the specimen lacks horny spines on the upper lip, it was nested in a clade with *Leptobrachium* (*Vibrissaphora*). Moreover, genetic divergence of the specimen from the other species was so great that it was assigned to an undescribed species, *Leptobrachium* sp. 5 from Xam Neua, northeastern Laos (Matsui et al., 2010). Although no additional specimens have been obtained, unique morphological traits of a single male strongly suggest its independent species status. Thus, I herein describe it as new to science.

MATERIALS AND METHODS

The specimen in question was fixed in 95% ethanol in the field, and later stored in 70% ethanol at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE). Measurements were taken to the nearest 0.1 mm mainly following Matsui (1984) for 21 morphological characters: 1) snout-vent length (SVL); 2) head length; 3) snout length; 4) snout-nostril length; 5) nostril-eye distance; 6) eye length; 7) tympanum-eye length; 8) tympanum diameter; 9) head width; 10) internarial distance; 11) inter-orbital distance; 12) upper eyelid width; 13) forelimb length; 14) first finger length; 15) outer palmar tubercle length; 16) inner palmar tubercle length; 17) tibia length; 18) foot length; 19) hindlimb length; 20) inner metatarsal tubercle length; and 21) first toe length. I followed the system proposed by Savage (1975) for the description of toe webbing states. The sex and maturity of the specimen were checked by minor dissection.

SYSTEMATICS

Leptobrachium (*Vibrissaphora*) *masatakasatoi* sp. nov.

Figs. 1, 2

Leptobrachium sp. 5 from Xam Neua,

northeastern Laos: Matsui et al., 2010, p. 265.

Etymology

The specific name is dedicated to the late Prof. Masataka Sato of Meijo University, Nagoya, Japan, who was an active entomologist and collected the type specimen of this new species.

Holotype

KUHE (Graduate School of Human and Environmental Studies, Kyoto University) 34396, a male from Phu Pan, Ban Saleui, Houaphan Province, northeastern Laos (20°12'N, 104°01'E, alt. 1750 m), collected between 16 and 21 August 2003 by Masataka Sato.

Diagnosis

The new species is assigned to the genus *Leptobrachium* by having a combination of the following characteristics: femoral glands present; oval, flat axillary glands present; inner palmar tubercle circular, not extending along first metacarpal; vomerine teeth absent; snout and/or dermal palpebral projections absent; rictal glands and ventrolateral glandular ridges absent (Hamidy and Matsui, 2010). The species is further assigned to the subgenus *Vibrissaphora* by the results of molecular phylogenetic analysis (Matsui et al., 2010) and by having a dorsoventrally broadly rounded snout. A small species of the subgenus, the unique adult male 55.2 mm in SVL; no spines on upper lip; tympanum indistinct; first finger longer than fourth; inner metatarsal tubercle smaller than first toe; 2½ phalanges of fifth toe free of webbing; skin nearly smooth, sparsely scattered with minute asperities, dorsum lacking distinct reticulation of ridges, flanks densely granulated; dorsum light brown with dark brown spots; flanks dark brown and reticulated with cream-colored spots; abdomen cream-colored with irregular brown markings.

Description of holotype (measurements in mm)

An adult male with large elongate testes with an SVL of 55.2; body robust, tapering to groin; head broad and depressed, length (23.7,



FIG. 1. Dorsal (A), ventral (B), and left lateral (C) views of the holotype of *Leptobrachium* (*Vibrissaphora*) *masatakasatoi* sp. nov. after preservation. Scale bar=10 mm.

42.9%SVL) slightly shorter than wide (24.7, 44.7%SVL); snout broadly rounded, sloping in profile, barely projecting beyond lower jaw; eyes large, not projecting from sides of head, length (7.6, 13.8%SVL) much shorter than snout length (11.0, 19.9%SVL); canthus sharp, straight; lore oblique, moderately concave; nostril lateral, below canthus, distinctly closer to eye (4.5, 8.2%SVL) than to tip of snout (5.8, 10.5%SVL); internarial distance

(5.0, 9.1%SVL) much shorter than interorbital distance (7.4, 13.4%SVL), internarial distance greater than length of upper eyelid (6.0, 10.9%SVL); no pineal spot; tympanum indistinct, diameter (3.1, 5.6%SVL) about two-fifths that of eye, and separated from eye by length subequal to tympanic diameter (3.0, 5.4%SVL); vomerine teeth absent; tongue heart-shaped, notched posteriorly, without papillae; no vocal openings.

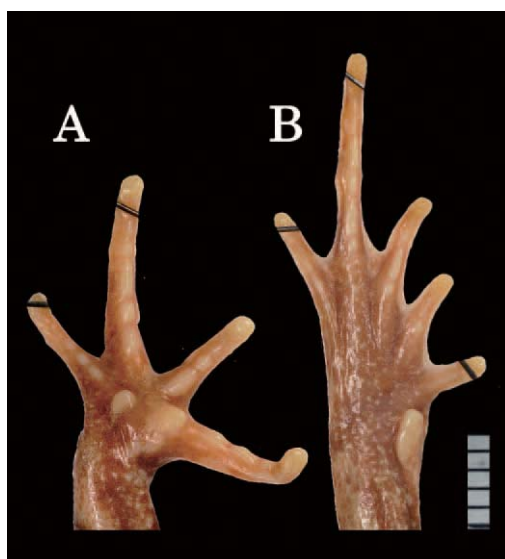


FIG. 2. Ventral views of right hand (A) and foot (B) of the holotype of *Leptobrachium* (*Vibrissaphora*) *masatakasatoi* sp. nov. after preservation. Scale bar=5 mm.

Forelimb long (41.4, 75.0%SVL) and slender; fingers moderately slender, unwebbed; relative length of fingers: $II < IV < I < III$; first finger much longer (7.2, 13.0%SVL) than second; tips rounded, slightly swollen; inner palmar tubercle large (2.5, 4.5%SVL), not extending onto first metacarpal and smaller outer palmar tubercle (1.9, 3.4%SVL); subarticular tubercles indistinct, replaced by low callous tissue; nuptial pads absent.

Hindlimb slender, relatively short (68.0, 123.2%SVL); heels not meeting when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching to posterior border of tympanum; foot (21.4, 38.8%SVL) longer than tibia (19.7, 35.7%SVL); relative length of toes: $I < II < V < III < IV$; toe tips similar to those of fingers; webbing poorly developed, formula: $I\ 2-2\ II\ 1\frac{3}{4}-2\frac{3}{4}\ III\ 2-3\frac{1}{2}\ IV\ 4-2\frac{1}{3}\ V$; toes with lateral fringes; subarticular tubercles obscure, but elongate, replaced by low callous tissue; inner metatarsal tubercle oval, length (3.0, 5.4%SVL) more than half the length of first toe (4.8, 8.7%SVL); no outer metatarsal tubercle.

Skin on head nearly smooth; upper lip with few minute asperities but lacking distinct spines; a low supratympanic ridge from eye to axilla; dorsum nearly smooth and sparsely scattered with minute asperities, some forming very weak short longitudinal ridges; slightly larger tubercles on dorsum posterior to sacrum, especially anterior to vent; strongly granulated around vent; flanks densely granulated with tubercles tipped by white asperities, throat and abdomen densely covered with minute white asperities, more granular at base of thigh; limbs with weak ridges dorsally, and smooth ventrally except for granulated base of thigh; a round, flat gland at median border of axilla behind arm; a flat, oblong femoral gland on distal half of posteroventral surface of thigh.

Color in alcohol

Dorsum of head and body light brown, with dark brown spots beginning on medial side of upper eyelid and continuing irregularly to vent; dark brown marking on canthus between nostril and eye, supratympanic fold beginning from posterior corner of upper eyelid; lips without marking; flanks dark brown reticulated with cream spots, markings on groin extending onto anterior face of thigh; abdomen cream-colored with irregular brown markings, especially on throat and abdomen; dorsum of limbs light brown marked with fine dark brown crossbars; posterior face of thigh dark brown with large white spots, continuous to bars across dorsal surface; femoral gland cream encircled by dark brown.

Comparisons

A previous phylogenetic estimate (Matsui et al., 2010) indicated that the most recent common ancestor of *L. (V.) masatakasatoi* is shared by either of *L. (V.) boringii* (Liu, 1945), *L. (V.) chapaense* (Bourret, 1937), or a group of other Chinese species of the subgenus *Vibrissaphora*: *L. (V.) liui liui* (Pope, 1947), *L. (V.) l. yaoshanensis* (Liu and Hu in Hu, Tian, and Wu, 1978), *L. (V.) leishanense* (Liu and Hu in Hu, Zhao, and Liu, 1973), and *L.*

(*V.*) *jiulongshanense* (Wei and Zhao, 1981). These species and subspecies are a sister group to *L. (V.) echinata* Dubois and Ohler, 1998 and *L. (V.) ailaonicum* (Yang, Chen, and Ma in Yang, Ma, Chen, and Li, 1983), and all of these are common sisters to *L. (V.) promustache* (Rao, Wilkinson, and Zhang, 2006) in the Subclade IV of Matsui et al. (2010), to the exclusion of all other species analyzed: the complex of *L. (L.) montanum* Fischer, 1885 and *L. (L.) abbotti* (Cochran, 1926), *L. (L.) gunungense* Malkmus, 1996, *L. (L.) hendricksoni* Taylor, 1962, *L. (L.) hasseltii* Tschudi, 1838, *L. (L.) smithi* Matsui, Nabhitabhata, and Panha, 1999, *L. (L.) lumadorum* Brown, Siler, Diesmos, and Alcala, 2010, and *L. (L.) nigrops* Berry and Hendrickson, 1963 (including *L. [L.] ingeri* Hamidy, Matsui, Nishikawa, and Belabut, 2012, and *L. [L.] kanowitense* Hamidy, Matsui, Nishikawa, and Belabut, 2012) of the subgenus *Leptobrachium*, and *L. (V.) hainanense* Ye and Fei, 1993, *L. (V.) mouhoti* Stuart, Sok, and Neang, 2006, *L. (V.) pullum* (Smith, 1921), *L. (V.) ngoclinhense* (Orlov, 2005), *L. (V.) xanthospilum* Lathrop, Murphy, Orlov, and Ho, 1998, and *L. (V.) banae* Lathrop, Murphy, Orlov, and Ho, 1998 of the subgenus *Vibrissaphora*. Genetic data indicate substantial levels of genetic divergence (>5.3% of uncorrected p-distance in partial 12S-16S rRNA genes) between *L. (V.) masatakasatoi* and all the other species of *Leptobrachium* (sensu lato).

Other published results (Brown et al., 2010; Hamidy and Matsui, 2010; Hamidy et al., 2011, 2012; Stuart et al., 2011, 2012; Wogan, 2012) and our subsequent mt DNA phylogenetic analysis of GenBank data of the species not analyzed in these studies (Hamidy and Matsui, unpublished data) indicate that *L. tagbanorum* Brown, Siler, Diesmos, and Alcala, 2010, *L. mangyanorum* Brown, Siler, Diesmos, and Alcala, 2010, and *L. rachinensis* Wogan, 2012 belong in the subgenus *Leptobrachium*. It was also indicated that *L. buchari* Ohler, Teynié, and David, 2004, *L. leucops* Stuart, Rowley, Tran, Le, and Hoang, 2011, and *L. xanthops* Stuart, Phimmachak,

Seateun, and Sivongxay, 2012 belong in the subgenus *Vibrissaphora* of Matsui et al. (2010), but in Subclade V and genetically remote from the present new species, while *L. huashen* Fei and Ye, 2005 is genetically very closely related to *L. (V.) chapaense* in the Subclade IV.

Thus it is pertinent to compare *L. (V.) masatakasatoi* with relatives in Subclade IV of Matsui et al. (2010) and several recently described species whose DNA data are not available. *Leptobrachium (Vibrissaphora) masatakasatoi* clearly differs from all species originally described as *Vibrissaphora* by the absence of spines on the male upper labium. From *L. (V.) chapaense*, the new species differs by having a male SVL of approximately 55 mm, finger formula II<IV<I, tibia 36%SVL, inner metatarsal tubercle smaller than first toe, and dorsal color without reticulated pattern (vs. males to 54 mm in SVL, finger formula II<I<IV, tibia shorter than 35%SVL, inner metatarsal tubercle as long as first toe, and dorsum posteriorly with dark reticulum in *L. [V.] chapaense*). *Leptobrachium masatakasatoi* differs from *L. (V.) huashen* by having a larger male body size of 55 mm in SVL, finger and toe tips swollen, and 2½ phalanges of fifth toe free of webbing (vs. males up to 51 mm in SVL, finger and toe tips not swollen, and 1½ phalanges of fifth toe free of webbing in *L. (V.) huashen*). The new species differs from *L. guangxiense* Fei, Mo, Ye, and Jiang in Fei, Hu, Ye, Huang et al., 2009 by having smaller male body size, 55 mm in SVL and indistinct tympanum (vs. males 62 mm or larger in SVL and tympanum distinct in *L. guangxiense*). Finally, *L. (V.) masatakasatoi* differs from *L. bompu* Sondhi and Ohler, 2011 by having a larger male body size of 55 mm in SVL, finger formula II<IV<I, shorter tibia 36%SVL, inner metatarsal tubercle smaller than first toe, and nearly smooth dorsum (vs. male SVL 47 mm, finger formula II<I<IV, tibia longer than 40%SVL, inner metatarsal tubercle as long as first toe, and dorsum covered with fine ridges forming a reticulum in *L. bompu*).

Range

Known only from the type locality, Phu Pan, Ban Saleui, Houaphan Province, north-eastern Laos.

Natural history

No record is available for the circumstances of collection. *Nanorana aenea* (Smith, 1922), *Raorchestes parvulus* (Boulenger, 1893), and *Tylototriton shanjing* Nussbaum, Brodie, and Yang, 1995 are the only species collected with the new species.

DISCUSSION

As recently as only one decade ago, the amphibian fauna of Laos was poorly known and an old reference by Bourret (1942) remained the major source of information. However, subsequent intensive surveys in the country have resulted in the discovery of many new species and new locality records in various amphibian lineages (e.g., Stuart and Heatwole, 2004; Stuart, 2005; Stuart and Bain, 2005; Stuart and Chan-ard, 2005; Rowley et al., 2010; Stuart et al., 2010a, b; 2012), although the diversity of vast areas in the country still remains imperfectly studied. Of *Leptobrachium*, Ohler et al. (2004) described *L. buchari* from southern Laos based on a single female specimen. In describing the species, the authors noted its green eye color. More recently, Stuart et al. (2012) described another species *L. xanthops* also chiefly based on its yellow upper iris.

As exemplified by these species from Laos, recent findings of new taxa of *Leptobrachium* (sensu lato) were partly helped by the eye color (Hamidy and Matsui, 2010), together with the application of molecular techniques (Matsui et al., 2010). Unfortunately, in the case of the present new species, it is impossible to determine the iris color because the specimen was fixed in ethanol, where the iris color disappears quickly, and has not been photographed in life. From the phylogenetic position in the mitochondrial tree and locality of collection, it would probably have a bicolored iris like many

other species from Vietnam and China whose range surrounds the type locality of the new species.

The supraspecific taxonomy of *Leptobrachium* (sensu lato) requires further studies. In relegating *Vibrissaphora* to the subgenus of *Leptobrachium*, Dubois and Ohler (1998) listed characters that are shared by *Vibrissaphora* and *Leptobrachium*. One of them was the presence of a Y-shaped yellow marking on the larval tail of both species of *Vibrissaphora* and of *L. chapaense*. Not knowing the paraphyly of *Leptobrachium*, Dubois and Ohler (1998) relied more heavily on the adult horny spines than the other characters, and did not move *L. chapaense* to *Vibrissaphora* based on a common larval trait. However, *L. chapaense* proved to be genetically nested in *Leptobrachium* (*Vibrissaphora*) in Matsui et al. (2010), suggesting that the classification of Dubois and Ohler (1998) should be reexamined.

Matsui et al. (2010) suggested that the upper lip is more broadened in *Leptobrachium* (*Vibrissaphora*) than in *Leptobrachium* (*Leptobrachium*). The present new species, while assigned to *Leptobrachium* (*Vibrissaphora*) molecularly, has a broadened upper lip supporting such a hypothesis. It is necessary to determine appropriate measurement points on the snout and to accumulate a sufficient number of data sets for many other taxa to test the wider applicability of this hypothesis. At the same time, the problem of reversed sexual size dimorphism emphasized in separating *Vibrissaphora* from *Leptobrachium* (Dubois and Ohler, 1998) should be also reassessed using additional samples of sufficient sizes for many taxa.

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